

Classification of Plasmodium Malaria and Plasmodium Ovale in Microscopic Thin Blood Smear Digital Images

Hanung Adi Nugroho, Aulia Darojatun, Igi Ardiyanto, Ratna L.B Buana

Department of Electrical Engineering and Information Technology,

Faculty of Engineering, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia

E-mail: adinugroho@ugm.ac.id; auli.daro@gmail.com; igi@ugm.ac.id; buana.ratna@gmail.com

Abstract— Malaria is one of the global diseases, which mostly found in eastern Indonesia. It is caused by *Plasmodium* parasite infection, with four type of common species that are *Plasmodium ovale* (PO), *Plasmodium Malaria* (PM), *Plasmodium falciparum* (PF) and *Plasmodium vivax* (PV). Malaria can be detected by taking a microscopic analysis from a patient blood sample. Although it is a gold standard of malaria identification according to the WHO, this method has a risk of miss diagnosis due to the human factors. This study proposed a classification method with morphological features of PM and PO in order to help the medical expertise in identifying the malaria parasite from a thin blood smear digital microscopic image. The data used are digital images that have been through the Region of Interest (ROI) determination process. Furthermore, the process followed by improving the morphological and feature extraction of shapes and colors. Based on these obtained features, the parasites are classified by using the multilayer perceptron method. From this study, we found that the classification system has the accuracy of 95%, the sensitivity of 93%, and the specificity of 97%.

Keywords— Malaria; *Plasmodium malaria*; *Plasmodium ovale*; multilayer perceptron; feature extraction; Computer Aided Diagnosis (CAD)

I. INTRODUCTION

Malaria is one of the global diseases, which is caused by the *Plasmodium* parasite transmitted by female Anopheles mosquitoes bite. In 2016, the World Health Organization (WHO) reported there are 216 million cases in the world, and 445.000 of them are written to be dead [1]. In the year of 2015, Indonesia has recorded 850 cases, which mostly found at Papua, Kalimantan and Sulawesi islands [2]. There are four types of *Plasmodium* parasite, which commonly found in Indonesia; they are *Plasmodium Malaria* (PM), *Plasmodium Ovale* (PO), *Plasmodium Vivax* (PV) and *Plasmodium Falciparum* (PF). These types have different resistance to particular medicine. Therefore, an accurate diagnosis is crucial. The negligence identification may hold up the patient recovery. Moreover, the severity process is expeditious and easy to spread especially in a tropical country.

Early and accurate detections are capable of reducing the number of mortality and the spread of malaria. WHO has suggested a diagnosis method, which is called the parasite-based diagnostic testing. One of the examples is diagnosis by using microscopic media. However, the weakness of this method may arise while it handled by inexperienced or an exhausted laboratory assistant. Another problem is the

limited equipment, which is not available in every medical facility, especially in the rural area. These problems may affect to the parasite type determination and lead to the wrong medical treatment.

Many aspects could support the early detection of malaria; one of them is the Computer Aided Diagnosis (CAD). CAD is a system that could support the medical practices in having a decision due to some human factor problems such as fatigue or fewer experiences. The backing system aim is to increase the diagnostic accuracy of malaria type from a thin blood smear sample.

Malaria is a global disease, which mostly hit the tropical countries. *Plasmodium Parasite* infects it in the human red blood cells. There are two hosts of *Plasmodium* in its life cycle, one is the female Anopheles mosquitoes as the definitive host, and the other is the human body or vertebrates as the secondary host [3].

The parasite life cycle shows in Fig. 1. Initially, the mosquito saliva on its bites to the human body transmits the parasite. Furthermore, the parasite entering the human body as sporozoite and infected the liver cells through the blood vessel. The parasite does the asexual reproduction and releases the merozoite in the bloodstream. Later on, each merozoite will infect the red blood cells and introduce another phase called the trophozoite or ring phase.

Furthermore, the parasite which infected the red blood cells develops into new phase namely schizont. After the schizont is mature enough, the cell core will release as merozoite and infected other red blood cells. The other infected cells will also develop as gametocytes cells. Gametocytes role as the asexual reproduction of the parasite life cycle [4].

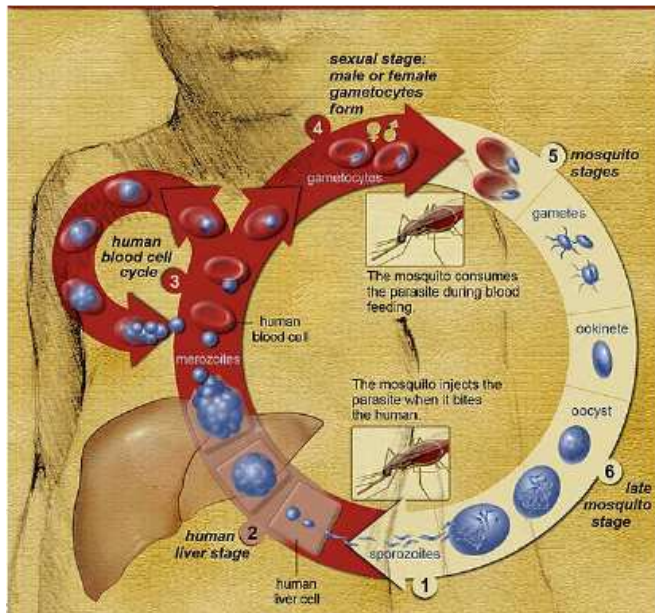


Fig. 1 Lifecycle of Malaria parasite [4]

The asexual life cycle developed in the definitive host, which spread by the female *Anopheles* mosquitoes bite. In PV and PO parasites, the trophozoite phase may develop into hypnozoite. The hypnozoite could live in the liver cells for months or even years, which may cause a relapse in a downturn immune to the human body [5]. The asexual reproduction phase may take 9 until 14 days for PF, 12 until 17 days for PV and 18 days for PM. While in the sexual life cycle, female and male gametocytes, which immigrate to the definitive host, will do the reproduction process and develop into an ookinete. Moreover, ookinete will flourish into oocysts and produce sporozoite, which will transmit to the secondary host. The sexual phase needs 26 to 28 days to complete one cycle [3].

There are several previous research conducted related to the CAD of malaria. Diaz et al. [6] proposed an approach on segmentation and classification method in order to quantify and classify the red blood cells which infected by PF. In the segmentation process, red blood cells are identified and segmented using luminance correction, pixel classification, and inclusion-tree representation. Another research by Tek et al. [7] is proposed a Bayesian pixel-classification on the segmentation stage in order to find the *Plasmodium* parasite candidate and applied some features such as shape, histogram and statistical moment features for the feature extraction stage in order to reduce the false-positive detection on the classification process. Elter et al. [8] improved a study by [9] on the detection method of PF in thick blood films. The development is containing two steps, which are high detection sensitivity to reduce the number of positive and applied the Support Vector Machine (SVM) for the classification process. The result showed that this

method could gain 97% sensitivity with false-positive detection on each image is 0.8.

In Bashir et al. [10] study, they used color intensity on the foreground object by manipulating the color from RGB channel to grey image with resolution 300 x 300 pixel. Furthermore, the morphological operation is applied to eliminate the noise. As a result, this study gained 99.68% of accuracy. Satria et al. [11] classified phase and species of PF and PV on thin blood smear image. In the pre-processing stage, contrast enhancement was applied to improve the image quality.

II. MATERIAL AND METHOD

The research data are digital microscopic images of thin blood smear samples, which infected by PO and PM parasites. These datasets obtained from Eijkman Institute Molekuler Biology of Rumah Sakit Cipto Mangunkusumo, central of Jakarta. The permission of these data utilization was approved by the pathologies and other related parties to use as the science and research interest.

The dataset is cropped on the Region of Interest (RoI) from the previous research [12] formerly. Afterward, they divided into three types of the dataset which are PO, PM and Not Parasite. Each dataset has specific staining and lighting with 250 x 250 pixels resolution. These three types of the dataset are used in the research, containing 34 PM images, 49 PO images 8 white blood cells images and 68 artifact images. Each image sample can be seen in Fig. 2.

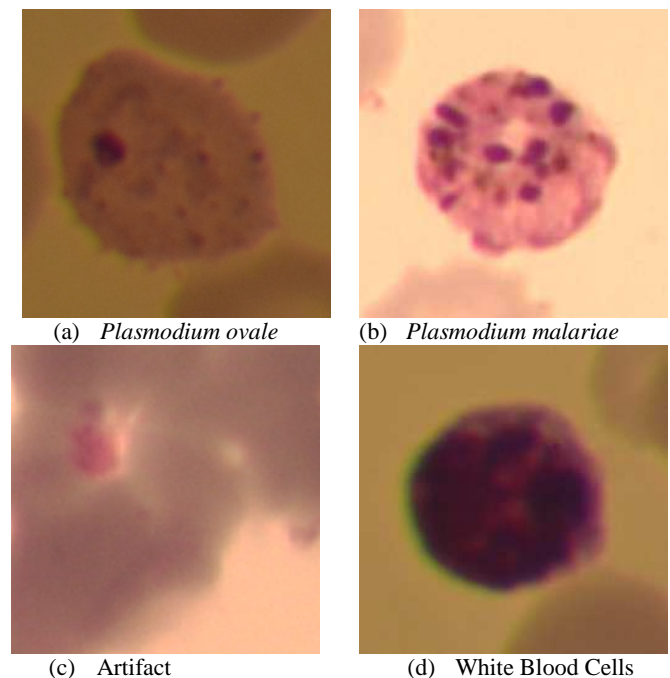


Fig. 2 Dataset image samples

As can be seen in Fig. 2, (a) PO and (b) PM has different image characteristic because they used disparate acquisition equipment even viewing by the same microscope. PO images are taken by Nikon D5200 camera, while PM images were taken by Olympus camera, which installed on the microscope.

Beforehand, the blood sample slides stained with a dye color called *Giemsa* in order to facilitate the object detection. Compare with another dye color such as *Leishman*, *Giemsa* is commonly used considering the price and the easier staining process [13].

The conducted research contains five main steps, namely pre-processing, segmentation, feature extraction, feature selection and classification as shown in Fig. 3. Each of these steps is explained in the following subsections.

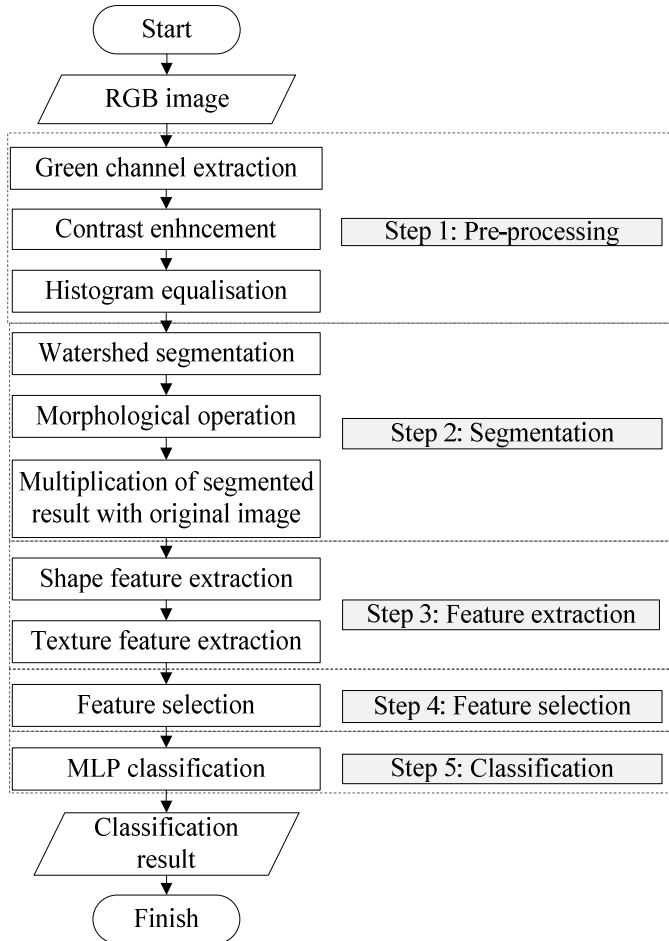


Fig. 3 Flowchart

A. Pre-processing

There are three parts arranged in the pre-processing stage, which are green channel extraction, contrast enhancement, and histogram equalization process. Compare with red and blue channels; green has the most contrast color between the object and background. Moreover, the contrast enhancement is conducted so that the object looks clearly in order to lighten the further process. The uneven histogram value created a poor image contrast. The pixels distribution in the histogram measures an image contrast quality. In a good contrast image, the distribution is uniform, which represents by no central peak or meadow. The contrast improvement can be done by histogram equalization, or precisely, the Gaussian distribution principle. This equalization application could be optimized for an image which strictly to Gaussian distribution [14]. This process changes the intensity value of a pixel by equally the pixel distribution

which called contrast stretching to represent in equation (1) [14].

$$o(i,j) = \frac{u(i,f) - c}{d - c} (L - 1) \quad (1)$$

with o as the before transformation pixel, u as the after transformation pixel, c is the maximum value of the input image and d is the maximum greyness of the image.

Contrast Limited Adaptive Histogram Equalization (CLAHE) is one of contrast enhancement methods with local functionality. In this method, the contrast improvement is limited to the homogeneous area by strengthening the noise. The thresholding is applied on the histogram gradient value. The threshold will crop the peak value on the homogenous area, and the equalization is obtained from the cumulative value with the less gradient. The cumulative histogram will generate a contrast image and reduce the noise [15].

In this research, histogram equalization is done with CLAHE algorithm to have the histogram value saturation of 1%, so that the histogram peak could be cropped' on 1% and equally distributed. Then, with the CLAHE algorithm, the histogram value is calculated in 8x8 window pixel. This is done to maximize the contrast enhancement on each pixel and to avoid the noise amplification on the image.

B. Segmentation

In the segmentation process, there are three steps which are segmented by using watershed, image enhancement with the morphological operation, and the multiplication of the segmentation result with the original image which used for the further process.

1) *Watershed segmentation*. Watershed segmentation is a segmentation method which divides the image into several different parts and represents it as topography relieve. This method illustrated as water characteristic which tends to flow to the lower area due to the gravity. In assumption, the topography has three different points, they are:

- the lowest point
- the particular point in which the water may drop
- the particular point which the water possibly dropped.

A group of pixels which have a minimum intensity in (b) condition called the valley shelter, while a group of pixels in the (c) condition called the watershed line. In other words, the watershed segmentation is the determination process in defining the watershed line [16].

2) *Morphological Operation*. In a digital image, morphological is a study of topology and structure of an object in the image. Morphological operation requires two inputs which are image and structuring elements (SE). The bases of morphological operation are dilation and erosion. The second derivation of these operations is opening and closing, while the third derivation is called white top hat and black top hat [17].

Opening operation is the derivation of erosion and dilation. In this operation, erosion is applied first, following with dilation. This operation may refine the object threshold, separate the united object and remove the object which lesser than the SE. The opening operation can be calculated by equation (2) [17].

$$X_B = (X \ominus B) \oplus B \quad (2)$$

Contrary with the opening operation, the closing operation initially applied the dilation followed with the erosion. This operation may refine the object on an image by tied the pieces and removed the small holes on the object. The closing operation can be calculated by equation (3) [17].

$$X^B = (X \oplus B) \ominus B \quad (3)$$

At first, the pre-processing image result changed into a binary image with a thresholding process. In the binary image, the distance between pixel with zero value to the nearest pixel of one value. Then, the distance values are grouped into some regions and place it into a labeled L matrix. From this matrix, the parasite region can be detected. Afterward, the morphological operation opening and closing are conducted to gain the segmented image which closes to the parasite shape. Then, this image is multiplied by the green channel image for the feature extraction process.

C. Feature Extraction

A feature extraction process is necessary to take the image descriptor from the segmentation result image due to the computer calculation. Based on Jain [18], there are some features can be used as a descriptor, they are spatial features, transform features, margin features, shape features, and texture features. In this research, two types of features are used, shape features and texture features.

1) *Shape Features*. Shape feature is physical structure and profile characteristics of an object in an image. This feature extraction can be applied after the image is segmented to an area and an object. The shape represented as contour, area, and transformation [18].

- *The area* is the number of pixels in an area which calculated by equation (4) below:

$$Area = \iint_{\mathcal{R}} dx dy = \int_{\partial \mathcal{R}} y(t) \frac{dx(t)}{dt} dt - \int_{\partial \mathcal{R}} x(t) \frac{dy(t)}{dt} dt \quad (4)$$

- *Circumference* is the margin length from an image which calculated by equation (5):

$$T = \int \sqrt{x^2(t) + y^2(t)} dt \quad (5)$$

- *Compactness* is the ratio between the square of the object circumference with the object area. This feature shows the object capability to fulfill the circle which

created by the object area. Compactness is calculated by equation (6):

$$\gamma = \frac{(Circumference)^2}{4\pi^2(Area)} \quad (6)$$

- *Roundness* is the ratio between the circle areas of an object with the square of the circumference. Roundness represents the similarity of the object to a circle which calculated by equation (7).

$$R = \frac{1}{\gamma} \quad (7)$$

- *Slimness* is the ratio between the object width with the object length which represents by equation (8).

$$Slimness = \frac{length}{width} \quad (8)$$

2) *Texture Features*. Texture feature provides the measurement to a smoothness, roughness, and regularity. The value is calculated by the statistical approach of histogram and pixel position [19]. The statistical approach has three kinds of level depend on the pixel intensity; they are first order, second order, and third order. GLCM (Gray Level Co-Occurrence Matrix) is one of the second orders of the statistical approach.

GLCM is a matrix has a dimension of columns and rows equal to the image greyness (G). Matrix element of $P(i, j / \Delta x, \Delta y)$ is a relative frequency between two pixels, which separated with a distance of $(\Delta x, \Delta y)$, in an area, with the intensity of i and j . $P(i, j / d, \theta)$ contains the second order of statistic probabilistic of greyness level transformation of i and j on particular distance movement of d , and the specific angle of θ . $M \times N$ of the image input consists of G level from 0 to -1, in the assumption of $f(m, n)$ is the intensity of m sample, n line over the area so that the matrix element can be calculated as equation (9) [19].

$$P(i, j / \Delta x, \Delta y) = WQ(i, j / \Delta x, \Delta y) \quad (9)$$

where:

$$W = \frac{1}{(M - \Delta x)(N - \Delta y)} \quad (10)$$

$$Q(ij | \Delta x, \Delta y) = \sum_{n=i}^{(N-\Delta y)} \sum_{m=1}^{(M-\Delta x)} A \quad (11)$$

From the image segmentation result of the binary image and green channel image, both shape and texture features are extracted. The binary image four features of shape are obtained such as circumference, area, roundness, and slimness. These features then grouped into some dataset as the feature selection input. Based on PM and PO characteristics on the little diagnosis guidance,

geometrically, PM and PO have a significant difference, PM with round red blood cell while PO with *amoebid* red blood cell. The second order of feature extraction histogram texture or GLCM is done on the image multiplication result. Since PM and PO have a significant distinction, some features such as contract, correlation, energy, and homogeneity are adequate to utilize as the classifier.

- *Contrast* is the spatial frequency value from an image and the measurement of the distribution of image matrix element which defines as equation (12).

$$Contrast = \sum_{n=1}^L n^2 \left\{ \sum_{|i-j|} GLCM(i, j) \right\} \quad (12)$$

- *Correlation* is the linearity measurement from some pixel couples which represent the information of the image linearity structure. Correlation is calculated by equation (13).

$$Correlation = \sum_{i,j} \frac{(i - \mu_i)p(i, j)}{\sigma_i \sigma_j} \quad (13)$$

- *Energy* is the indicator of image uniformity which calculated by equation (14).

$$Energy = \sum_{i,j} p(i, j)^2 \quad (14)$$

- *Homogeneity* is the measurement of image uniformity based on the greyness. The bigger value shows that the image is homogeneous, which can be calculated by equation (15).

$$Homogeneity = \sum_{i,j} \frac{p(i, j)}{1 + |i - j|} \quad (15)$$

D. Feature Selection

This process is necessary to eliminate features, which not bring a significant effect to the result. More numbers of features cannot ensure a better classification; on the contrary, it may increase the computation process and may reduce the accurate result. Wrappers method is an evaluation to quest the most significant feature by using a particular classifier. Best first search forward method started from an empty feature set. If the feature is considered relevant, it will be selected as the selection result and will not be eliminated. This process repeated until there is no more significant feature.

E. Classification

In this study, Multilayer Perceptron (MLP) is conducted as the classification algorithm which is used an artificial neural network which has a supervised characteristic. It is

commonly used in non-linear cases. MLP has several hidden layers containing input and output layer which illustrated in Fig 4 [20].

This research goal is to classify the image into three different classes; they are not a parasite, PM class, and PO class. Four numbers of hidden layer are chosen with unipolar sigmoid as the activate function. Later, the classification result is used as the MLP input to do weighting. In this research, back propagation multilayer perceptron with 500 epochs and 0.3 of learning rate are used. Training and testing data are split by using k fold cross-validation.

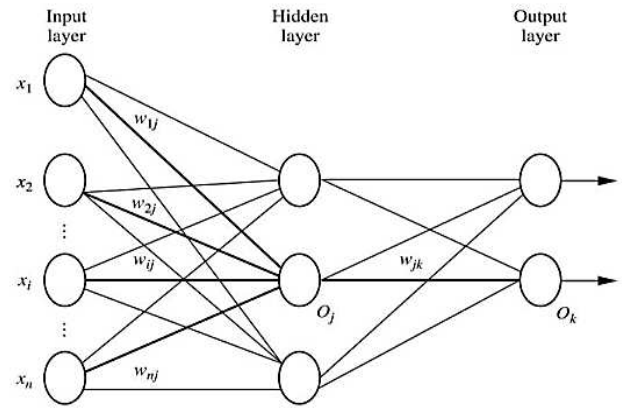


Fig. 4 Multilayer perceptron diagram [20]

F. Validation

The validity of a classification result can be evaluated by calculating the number of true data on the precise class (true positive). It also computed the number of true data which is not belong to the class (true negative). It also examined, the number of false data in the precise class (false positive). Ultimately, the validity also evaluate the number of false data which is not belong to the class (false negative). These data create a confusion matrix which shown in Table I.

TABLE I
CONFUSION MATRIX

Data Class	Classified as Positive	Classified as Negative
Positive	True Positive (TP)	False Negative (FN)
Negative	False Positive (FP)	True Negative (TN)

In a multiclass case, the validation represents by the accuracy, sensitivity and specificity calculation which formulated by equation (16), (17) and (18) respectively.

$$accuracy = \frac{TP + TN}{TP + FN + FP + TN} \quad (16)$$

$$sensitivity = \frac{TP}{TP + FN} \quad (17)$$

$$specificity = \frac{TN}{FP + TN} \quad (18)$$

III. RESULT AND DISCUSSION

A. Pre-processing

Fig.5 shows the pre-processing result start from (a) original image, (b) green channel image, (c) image which cropped on the green channel threshold, and (d) histogram equalization result. The result shows that the green channel is able to create a significant border between object and the background. However, both PM and PO images are still blurry. After contrast enhancement is conducted, the image is brighter and the parasite candidate is emerged between the other red blood cells. The CLAHE process made the background more blurry so that in schizont stage, where the core cell is dominant, both PM and PO appear more contrast and clear.

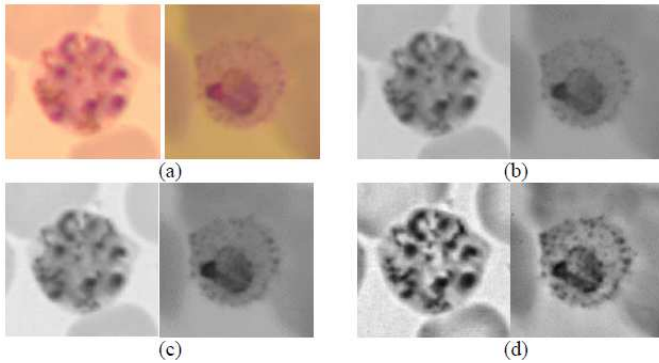


Fig. 5 PM and PO images of (a) original image, (b) green channel image, (c) cropped by the thresholding, and (d) CLAHE image result

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B. Segmentation

Fig. 6 (a) to (c) show the segmentation result for PM, which is (a) segmentation result with watershed, (b) morphological operation result, and (c) the multiplication result of green channel and the original image. As seen in the figure, the watershed could not give the perfect parasite shape. There are some holes found in the middle of the parasite candidate. The parasite shape is tending to resemble as circle or oval. Therefore, the morphological operation of the disc is applied. The result in Figure 4.3 (d) shows that PM characteristic is more likely to a circle, while PM tends to be an oval. Last, the multiplication of the green channel image and the original image are conducted as the input of the feature extraction process.

C. Feature Extraction

In this study, the wrappers method is used in forwarding direction with MLP as the classifier simulation. As a result, there are five features which contribute significantly; they are contrast, correlation, homogeneity and area, which are used as the input of the classification process.

D. Classification

There are two steps in the classification process, training, and testing. *K*-fold cross-validation with 10 fold is applied to divide the data for both processes. All data are divided into 10 groups, one group for the training process, while the rest is used for the testing process. Table II shows the confusion matrix of the classification result. From this confusion matrix, the value of accuracy, sensitivity, and specificity of the system could be calculated as shown in Table III.

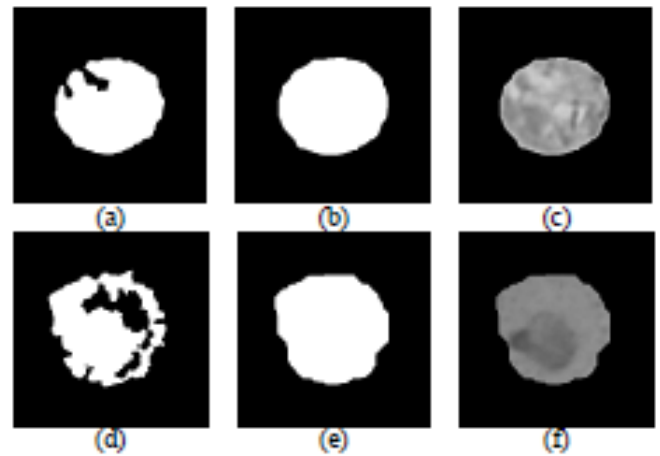


Fig. 6 PM image of (a) segmentation result, (b) morphological operation result, (c) multiplication result; and PO image of (d) segmentation result, (e) morphological operation result, (f) multiplication result;

TABLE II
CONFUSION MATRIX RESULT

OUTPUT	INPUT			
	Parasite Types	PM	PO	Not Parasite
	PM	32	1	2
	PO	1	47	1
	Not Parasite	1	1	73

TABLE III
ACCURACY, SPECIFICITY AND SENSITIVITY RESULT

Parasite Types	Sensitivity	Specificity	Accuracy
PM	91.42%	97%	95.45%
PO	94%	97%	96%
Not Parasite	96%	97%	96%

IV. CONCLUSIONS

The result shows that the watershed for segmentation process is relevant for both PM and PO which ROI already choose. Moreover, the wrappers for feature selection capable of increasing the classification performance of PM and PO. At last, the MLP in the classification process is adequate to differentiate between PM and PO, and a Non-Parasite object such as leucocytes and artifact. In future, this

study result can be used as one of the CAD systems to classify malaria species.

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